Clinico-Pathological Conference

Disseminated cryptococcal infection despite treatment for cryptococcal meningitis

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Case Reports *(Dr R F Miller)*

Case 1

A 25 year old Caucasian heterosexual man was found to be HIV I antibody positive in July 1989, when he presented to another hospital with cryptococcal meningitis. At this time his CD4+ count was 190 (normal range = 350–2,200)/mm^3. Investigations showed normal urea and electrolyte levels and liver function tests; CT of the head was normal. Blood and urine culture was negative, India ink staining of CSF was positive for *Cryptococcus neoformans* and this organism was cultured from CSF. The CSF protein was 0·42 g/l, 30 lymphocytes/mm^3 were seen and the CSF/plasma glucose ratio was 2·2/5·6 mmol/l. Cryptococcal antigen (CRAG) titre, using latex agglutination, was 1 in 2052 in both CSF and in blood.

The patient was treated with intravenous amphotericin B, 1 mg/kg/day for four weeks and responded well clinically; at repeat lumbar puncture performed after four weeks treatment the CSF CRAG had fallen to 1 in 256. Subsequently the patient was maintained on oral fluconazole 400 mg daily. This was discontinued in October 1989 because of abnormal liver function tests. The patient rapidly developed headache and was admitted in November of that year. Investigations then showed normal urea and electrolyte levels and liver function tests and negative blood cultures. Blood CRAG was 1 in 5000, CSF CRAG was 1 in 1026. Cultures of CSF were positive for *C neoformans*. The patient was given a further course of intravenous amphotericin B for four weeks and subsequently a Hickman line was inserted; he then received 0·5 mg/kg of amphotericin intravenously once weekly, as maintenance therapy and began Fansidar two tablets once weekly for primary prophylaxis against pneumocystis pneumonia.

He remained well until April 1990 when he had a further relapse of meningitis whilst receiving maintenance, which was again treated with four weeks of amphotericin B at a dose of 1 mg/kg/day. He then went back onto weekly maintenance amphotericin at the doses given above.

He was admitted to the Middlesex Hospital in August 1990 with a four day history of generalised headache and nausea. On examination there was slight ataxia but no other neurological signs were evident. Urea and electrolyte levels and liver function tests were normal, CT of the head showed atrophy only; in particular there was no hydrocephalus and no cryptococcomas were seen. The CD4 count was 40/mm^3. Blood cultures were negative for fungi, acid and alcohol fast bacilli (AAFB) and bacteria. At lumbar puncture there were 20 lymphocytes/mm^3, CSF/plasma glucose ratio was 1·7/5·1 mmol/l and CSF protein was 0·69 g/l. Gram, auramine and India ink stains of CSF were negative and culture for bacteria and mycobacteria, viruses and fungi were all negative. Syphilis and toxoplasmosis serology were negative in CSF and blood. The CRAG titre in CSF was 1 in 256 and in blood was 1 in 2052. The Hickman line entry site was painful and red and there was crusting sero-purulent discharge from this site. Culture from the skin site (and from the catheter tip) grew *Staphlococcus aureus* (sensitive to flucloxacillin). The patient was treated with intravenous flucloxacillin and the Hickman line was removed.

After discussions between his wife and his carers the patient declined further treatment with amphotericin and instead chose to receive oral fluconazole 800 mg once daily. He was transferred to a hospice where he developed slurred speech, poor balance and neck stiffness. Pre-terminally he had photophobia and muscle tenderness. Terminally he developed bronchopneumonia and died four weeks after transfer to the hospice. A perimortem serum CRAG was > 1 in 10 000.

Pathology (Dr S B Lucas)

The pneumonia involved both lungs (weights 900 g and 980 g) with focal haemorrhagic lesions, macroscopically resembling *aspergillus* pneumonia. The liver was enlarged at 2150 g, the spleen appeared normal and weighed 170 g. All lymph node groups were atrophic except the retroperitoneal nodes which were soft and up to 2 cm diameter. The brain weighed 1540 g and the leptomeninges looked opaque. On slicing there were no abnormalities in the brain. The spinal cord was unremarkable.

Histopathology revealed a haemorrhagic pneumonia due to vascular damage from invasion of pulmonary arterial walls by Gram negative bacilli. Mycobacteriosis was seen in some small bowel villi and in the intra-abdominal nodes. The portal tracts of the liver were inflamed but there were no features of drug-induced hepatitis. The adrenals had minor cytomegalovirus infection. Cryptococcal infection was seen diffusely in the cerebral and spinal meninges; unlike the appearances in untreated cases, most of the yeasts were within macrophages rather than

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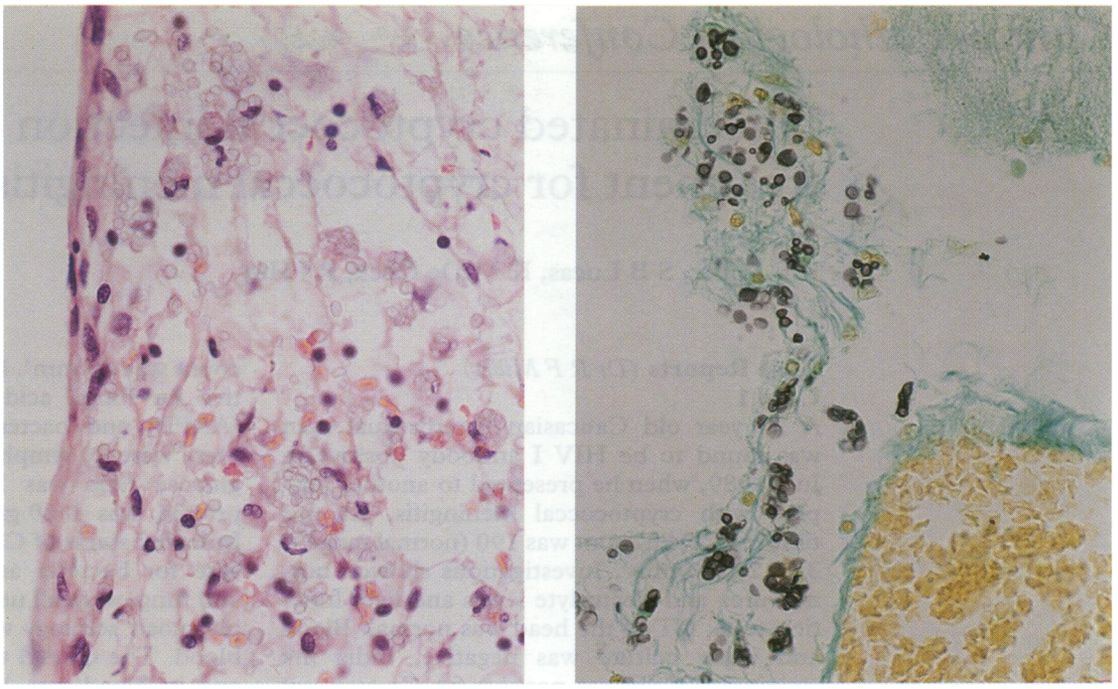

Figure 1 Case 1. The meninges, showing Cryptococcus neoformans yeasts within macrophages. Magnification × 250; left panel haematoxylin and eosin, right panel Grocott's methenamine silver stain.

lying free, but budding was evident (fig 1). The intra-abdominal notes contained some cryptococcal yeasts.

Conclusions of autopsy

Major causes of death were (1) Recurrent cryptococcosis, (2) Necrotising Gram negative pneumonia. Minor pathologies were 1. Disseminated *M. avium-intracellulare* infection 2. Cytomegalovirus adrenalitis.

Case 2 (Dr R F Miller)

A 49 year old Caucasian homosexual antiques dealer, who was a heavy drinker of alcohol (> 40 units per week) and a smoker of 40 cigarettes per day, was found to be HIV antibody positive in 1990. He remained well and in November 1992 his CD4 count was 430/mm^3. He was admitted to this hospital in December 1992 with a two month history of weight loss (5 kg), fever and sweats and a two week history of increasing exertional dyspnoea. Examination revealed no focal abnormalities on general and neurological examination.

Investigations revealed hyponatraemia, (sodium = 131 (normal range = 135–142) mmol/l), other electrolyte levels were normal, alkaline phosphatase level was 620 (normal range < 180) iu/l; other liver function tests were normal. Cultures of blood, stool and urine were negative for bacteria, fungi and mycobacteria. Arterial blood gases taken with the patient breathing room air, showed PaO_2 = 4·9 kPa, $PaCO_2$ = 3·9 kPa; a chest radiograph showed marked bilateral interstitial shadowing.

He was thought to have *Pneumocystis carinii* pneumonia and was treated with high dose intravenous co-trimoxazole and methylprednisolone. Despite this treatment he deteriorated further and required transfer to the intensive care unit for continuous positive air way pressure ventilation via a face mask. Subsequently he made a rapid recovery. Bronchoscopy with bronchoalveolar lavage was performed during recovery and this was negative for *P carinii* and the auramine stain was negative; subsequently acid-fast bacteria were grown after ten days culture. Rifampicin, isoniazid, pyrazinamide and ethambutol in conventional doses were begun, pending the results of culture and identification. There was a rapid increase in arterial oxygenation together with clearing of the chest radiograph. After six weeks of quadruple therapy the bacteria were identified as *Mycobacterium tuberculosis*, which was fully sensitive to first line drugs. After two months quadruple therapy treatment was rationalised to rifampicin and izoniazid only and the patient began co-trimoxazole as prophylaxis against pneumocystis pneumonia. In May 1993 he was well and his CD4 count was 120/mm^3.

He was admitted in June 1993 with a two weeks history of increasing confusion, vague bifrontal headache, nausea and occasional vomiting, together with fatigue and malaise. On examination he was confused, disorientated in time and place, had extensor plantars but no other focal signs were evident; specifically there was no neck stiffness. General examination showed no lymphadenopathy, no hepatosplenomegaly and the chest was clear on auscultation. Investigations revealed haemoglobin = 10·3g/dl, WBC = 4·3 × 10^9/l, serum sodium = 133mmol/l, serum potassium 3·1 (normal = 3·3–4·5)mmol/l, calcium = 2·02 mmol/l, albumin 28g/l, corrected calcium = normal. The alkaline phosphatase and bilirubin were normal but the aspartate aminotransferase = 122 (normal range = 11–55) iu/l. A chest radiograph was normal.

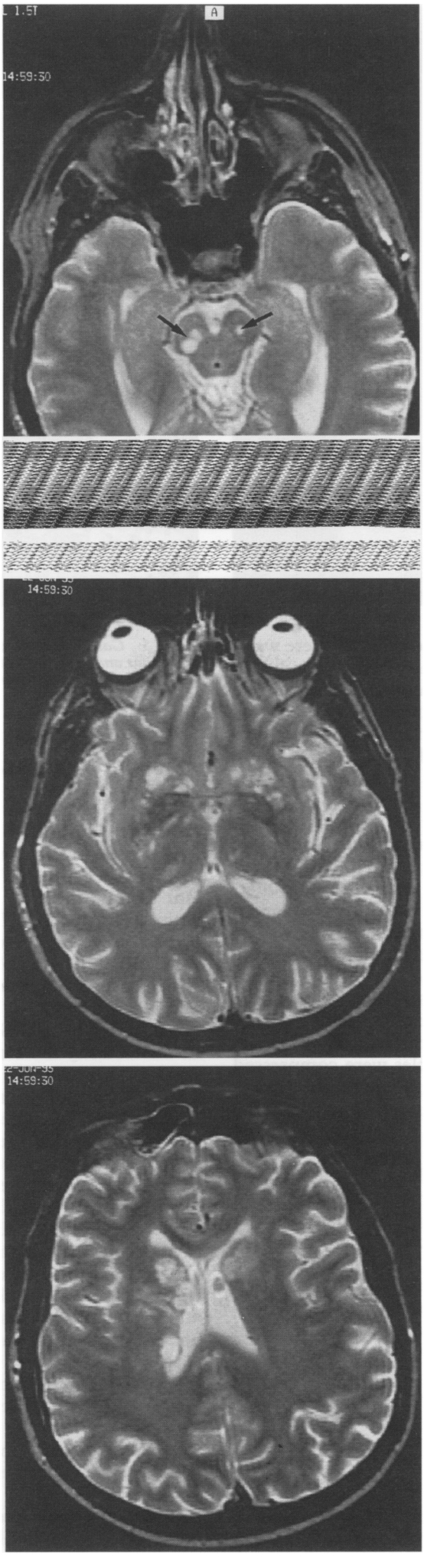

Figure 2 Case 2. MR scan of head, axial plan. T2 weighted images (CSF appears white) (a) at level of cerebral peduncles showing cryptococcomas (arrows) with uniform hyper intense signal (b) at level of mid thalamus, further cryptococcomas are seen (c) at level of internal capsule. Bilateral caudate nuclei lesions are demonstrated; in addition a cryptococcoma is seen impinging on the posterior horn of the lateral ventricle. There is co-existent mild cortical atrophy and background white matter signal change in the medial temporal lobe and posterior parietal lobe, suggesting HIV encephalopathy.

MRI of the head showed localised areas of increased signal (these were non-gadolinium enhancing), in the brainstem and basal ganglia (fig 2). An EEG showed diffuse non-specific abnormalities. Lumbar puncture showed 20 lymphocytes/mm^3. The CSF protein was 0·46 g/l and the CSF/plasma glucose ratio was 2·9/5·3, Gram and auramine stains were negative, India ink and mucicarmine stains showed *C neoformans* (fig 3). Toxoplasma and syphilis serology was negative in blood and CSF. Culture of blood and CSF was negative for bacteria and mycobacteria but positive for *C neoformans*. The CRAG titre in CSF was 1 in 131 000 and in blood was also 1 in 131 000. Culture of CSF and DNA amplification was negative for *Herpes simplex* type 1, varicella zoster virus and cytomegalovirus.

Amphotericin B 0·75 mg/kg/day was given. Despite this treatment the patient's condition worsened, he became increasingly confused and was unable to swallow, so a nasogastric tube was placed and enteral feeding was begun. The amphotericin was increased to 1·5 mg/kg/day and flucytosine 150 mg/kg/day was started. This had little impact on his symptoms and he became anaemic (haemoglobin = 7·3 g/dl) and the serum creatinine rose to 253 μmol/l. The patient was transfused with 4 units of blood. At this point, repeat blood cultures were still positive for *C neoformans*. After three weeks treatment with amphotericin B and flucytosine repeat MRI showed an increase in the size of the basal ganglia and brainstem lesions; none showed a diminution in size in response to treatment. Serum CRAG titres remained elevated at 1 in 64 000. Repeat lumbar puncture was not performed because of the risk of coning. After a further two weeks of treatment (that is, a total of five weeks treatment), repeat MRI showed further increase in size in the lesions. At this point the patient had received a total of approximately 2 g of amphotericin B. Treatment was changed to fluconazole 1,200 mg intravenously once daily (because of liver inducing effects of rifampicin a higher dose of

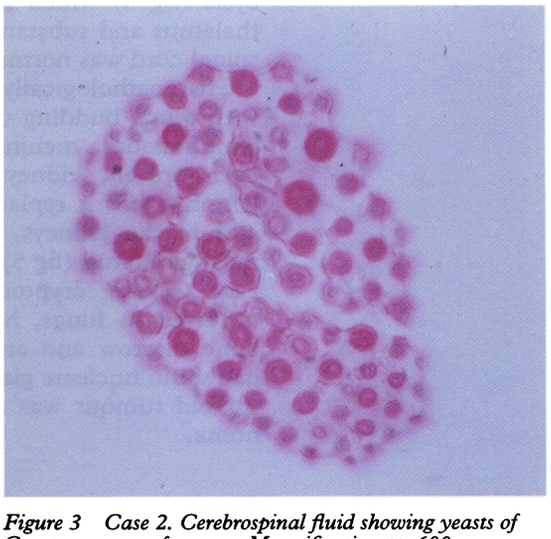

Figure 3 Case 2. Cerebrospinal fluid showing yeasts of Cryptococcus neoformans. Magnification × 600, mucicarmine stain (Courtesy Dr Gabrijela Kocjan).

Figure 4 Case 2. Coronal slice through brain, showing multiple microcysts of cryptococcal invasion in basal ganglia and white matter.

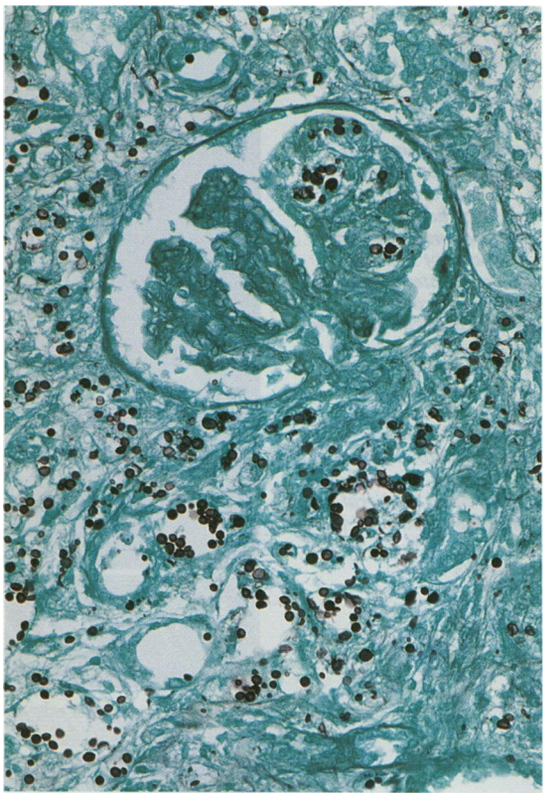

Figure 5 Case 2. Kidney with cryptococcal yeasts in a glomerulus and in the interstitium. Magnification × 100, Grocott's methenamine silver stain (yeasts are stained black).

fluconazole was used). Despite this there was a steady deterioration in the patient's condition, with a further rise in serum creatinine and he became anuric. Terminally he became increasingly confused and hypotensive, ultimately developing cardiogenic shock from which he died.

Pathology (Dr S B Lucas)

Externally the patient was wasted. Both heart and lungs (weights 430 g and 410 g) appeared normal. The liver was of normal size (1800 g) and appearance, but the spleen was enlarged (510 g) and showed white pulp atrophy. The mesenteric and para-aortic lymph nodes were 5 mm in diameter, white and firm. The left lobe of the thyroid contained a fleshy encapsulated tumour. The brain (weight 1410 g) and meninges were externally normal, but on slicing, the lateral ventricles were compressed to slits. The basal ganglia contained microcysts (fig 4) which extended down into the thalamus and substantia nigra. Externally the spinal cord was normal.

Histopathologically, florid cryptococcosis, with much budding of yeasts, was present in lymph nodes, meninges, brain parenchyma, liver, spleen, kidney and adrenals. In the lymph nodes it replaced the lymphoid tissue and in the kidneys, yeasts were present in most glomeruli (fig 5) and in the interstitium. Less severe cryptococcal infiltration had occurred in lungs, heart, thyroid, pancreas, bone marrow and anus. The cerebrum also had multi nucleate giant cell encephalitis. The thyroid tumour was a benign follicular odenoma.

Conclusions of necropsy

Major cause of death was (1) Disseminated cryptococcosis. Minor pathologies were (1) giant cell encephalitis, (2) follicular adenoma of thyroid.

Discussion (Professor R Hay)

Cryptococcus neoformans is an unusual yeast, in that it has a capsule. This capsule is an important anti defence mechanism and is antiphagocytic. In HIV positive patients the highest prevalence rates are seen in Northern Thailand; in Chiang Mai 26% of patients with AIDS have cryptococcal infection, a colossal figure and in parts of Zaire the prevalence in individuals with AIDS is approximately 14%. In this country the Public Health Laboratory Service estimates the figure to be 3–3·8%. The spectrum of disease produced by *C neoformans* in HIV infected individuals is very different from that seen in patients immunosuppressed from other causes; as we saw in Case 2, patients frequently have positive blood cultures (which is rare in non-HIV infected individuals). Dissemination of infection around the body is common; thus serum CRAG titres are much higher than CSF titres, whereas in other populations it is generally the other way round. This fact is clinically useful, because it means measuring CRAG in blood is an appropriate way of screening for disease as well as monitoring progress and response to therapy. With effective treatment CSF CRAG titres fall first and the serum antigen changes lag behind those in the CSF. Likewise treatment regimens are very different in the two groups. For example there is little evidence that fluconazole is curative for cryptococcal meningitis in non-HIV infected patients.

Features associated with a poor prognosis in HIV positive patients with cryptococcal meningitis

a *at presentation*
- Hyponatraemia
- Elevated CSF opening pressure > 30 cm H_2O
- Positive India ink (or equivalent) stain of CSF
- CSF CRAG titre > 1 in 10,000
- Blood, urine, induced sputum (or bronchoalveolar lavage) culture positive
- Relapse of infection
- Combination of:
  i. abnormal mental state
  ii. CSF CRAG titre ⩾ 1 in 1024
  iii. CSF white cell count < 20/mm³
- Cryptococcomas on cerebral CT or MR imaging
- CD4 count < 200/mm³

b *following treatment*
- CSF CRAG titre > 1 in 8

Most data are derived from the HIV positive patient population and fluconazole is less often used as first line treatment in non-HIV infected patients.

Data also suggest that following a primary "induction" course of treatment, the chances of achieving a cure are very low in HIV infected patients with cryptococcal meningitis, probably 30% or less, which is in marked contrast to other immunosuppressed patients. So the strategy employed most widely involves an induction course of treatment followed by long-term maintenance therapy.

In the first case fluconazole was stopped because of abnormal liver function tests. Fluconazole has a good safety record. As it is an azole it is a possible cause of hepatotoxicity, but this is a rare occurrence. It is unclear in this case whether the fluconazole was relevant to liver dysfunction but stopping the fluconazole was a reasonable decision. The other interesting aspect of this case was the initial treatment. Induction therapy used iv amphotericin B for 4 weeks (an appropriate choice). There is considerable debate as to whether flucytosine should be added to amphotericin B in the initial phases of treatment in HIV infected patients. This debate has not been resolved.[1-4] In contrast, in non-HIV infected individuals use of flucytosine in addition to amphotericin B has been demonstrated to be superior to amphotericin B alone.[5] My personal preference is to use combination therapy with amphotericin B and flucytosine, but there are no trial data to back this view. I would concur with the decision to continue maintenance therapy with oral fluconazole 400mg/daily.

Returning to the problem of the use of fluconazole—the CSF antigen titres were high but not grossly elevated (only CSF values of > 1 in 10 000 carry a poor prognosis), CSF and blood CRAG titres were similar, suggesting he did not have rapidly disseminating disease. This would have been an appropriate time to do sensitivity tests as it might have helped in deciding whether to change to maintenance intermittent iv amphotericin B (which is what happened) or switch to an alternative form of amphotericin B, for example, one of the liposomal or lipid associated formulations or use a higher dose of fluconazole or change to itraconazole. There are few data on the use of itraconazole for the treatment of cryptococcal meningitis in AIDS patients. However, 400 mg/day of itraconazole is effective in some patients as primary therapy and can be used as long term maintenance; it appears to be less effective than amphotericin B and flucytosine.[6] Sensitivity testing is difficult to carry out but fluconazole resistant cryptococcal infections may occur. Amphotericin B resistance is rare.

In conclusion, in Case 1 there is progression of cryptococcal infection in a patient with a rapidly progressive decline in CD4 count (which is indicative of decline in the primary defence against this disease). Given this scenario the prognosis was very poor at the outset, even though there was apparent clinical control of cryptococcal infection, in the initial stages.

The second case contrasts with the first, as cryptococcal infection continued to progress despite therapy. The patient presented, even in the initial stages, with a very high serum cryptococcal titre. This raises the question, with hindsight, as to whether the disease could have been detected earlier? If it had been diagnosed earlier, would starting treatment at this stage have made any difference? In this case with well established infection there was no response to primary therapy. The screening test for cryptococcal infection—the latex agglutination test or CRAG—is cheap and easy to perform. False positive results are quite rare but patients with circulating rheumatoid factor may have a "false positive" result (but this can be controlled by pre-treatment of serum with reducing agents, such as dithiothireitol, or by protease digestion).

At the time of diagnosis patient 2 had three poor prognostic signs, hyponatraemia, very high CSF and serum antigen titres and positive blood cultures (table).[1 3] I would also have used amphotericin B with flucytosine from the outset.[7] In non-HIV infected patients with cryptococcal meningitis and cryptococcomas, solid tumours of fungus, with little inflammation, results of treatment are not good. Studies have shown that even though a patient may be comparatively well, these lesions take a long time to clear. It is likely that in patients with cryptococcomas there is a problem of drug penetration into the gelatinous fungal mass of the cryptococcoma.

If the combination of amphotericin B and flucytosine is used it is important to remember that amphotericin B reduces the renal excretion of flucytosine. Flucytosine suppresses bone marrow function, which is directly related to serum drug levels. Looking at case 2, it would have been sensible to use a lower dose of amphotericin B, say 0·6–0·8 mg/kg/day, if one was also giving flucytosine. Monitoring serum flucytosine levels might have helped avoid the marrow toxicity that occurred in this case. Toxicity from flucytosine is usually manifest in the second week of therapy, so a drug level taken after 3 days of flucytosine and another towards the end of the first week, would enable dose adjustments to be made. The dose may need to be reduced at this stage, as this is the point at which

amphotericin leads to reduced renal clearance of flucytosine. In Case 2 maximum dose levels of both drugs were used and aside from toxicity there was no clinical response. One ought to consider if there would have been any benefit from switching to liposomal amphotericin B. There are no comparative data showing treatment advantages for liposomal compared to conventional amphotericin B in cryptococcosis.[8] It was therefore reasonable to try high dose of fluconazole, as amphotericin B and flucytosine had failed.

It is often unclear why patients with some infectious diseases develop relentless progression and die; clearly in HIV infected individuals with opportunistic infections, there is also progressively severe underlying immunosuppression. In the case of cryptococcal infection there is also evidence to suggest that the capsule of *C neoformans* can itself modulate some forms of immune function. It can reduce B cell activity and impair macrophage uptake of cryptococcal cells.[9]

Dr KM De Cock

Seeing these two cases presented I have the feeling that cryptococcal infection in the context of HIV infection is an incurable disease.

Professor R Hay

With the strategy of an initial treatment phase and subsequent maintenance therapy, most HIV infected patients survive their cryptococcal infection, but patient 2 undoubtedly died because of cryptococcosis, despite maximal therapy. In a study carried out by the Public Health Laboratory Service, which examined cryptococcal antigen data from HIV positive patients with cryptococcal meningitis who had received long term maintenance therapy, less than 5% became antigen negative. This suggests that whatever regimen is used, even if the patient becomes asymptomatic, there is persistence of cryptococcal antigen, presumably indicating the continued presence of organisms.

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